

Proteinase K protection assay

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 An abbreviated version of this protocol was published in eLIFE in Nov 2015

Translocation of interleukin-1 β into a vesicle intermediate in autophagy-mediated secretion

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Detailed protocol

1. Seed cells (e.g. HEK293T cells) in a 10cm dish and culture cells to confluence (1×10^7 cells).
2. Collect the cells by a scraper, followed by centrifugation at $600 \times g$ for 5 min at 4°C .
3. Wash the cells twice with ice-cold PBS, and suspend them in 250 μL (2.5 times the volume of cell pellet) of HB1 lysis buffer.
4. Homogenize the cells with ~20 strokes using a 1 mL syringe with 22-G needle. Stain 2 μL cell lysate with trypan blue and visualize through a microscope. If less than 90% cells are lysed, add more strokes. (Note: Times of strokes varies among different types of cells.)
5. Centrifuge the sample at $1000 \times g$ for 10 min at 4°C to recover the postnuclear supernatant (PNS).
6. Centrifuge the PNS at $25,000 \times g$ for 10min at 4°C to collect the 25K membrane pellet.
7. Wash membrane pellet with 500 μL B88 buffer and centrifuge at $25,000 \times g$ for 10min at 4°C .
8. Resuspend membrane pellet in 100 μL of B88 and divide into three 30 μL of aliquots. Of the three aliquots, one is control, the other two are incubated with 10-30 $\mu\text{g/mL}$ proteinase K (1mg/ml stock) with or without 0.5% Triton X-100 (10% stock) on ice for 30 min.
9. Stop the reactions by adding 3 μL 100 $\mu\text{g/mL}$ AEBSF/PMSF on ice for 10 min.
10. Add boiled 3 \times SDS loading buffer and boil the sample for 10 min followed by Western blot analysis.

HB1 lysis buffer

20mM HEPES-KOH, pH 7.2
1mM EDTA
400mM sucrose
Protease inhibitors
Phosphatase inhibitors
0.3 mM DTT

B88 buffer

20mM HEPES-KOH, pH 7.2
250mM sorbitol
150mM potassium acetate
5mM magnesium acetate

Reference

1. Min Zhang, Lei Liu, Xubo Lin, Yang Wang, Qing Guo, Ying Li, Shulin Li, Yuxin Sun, Di Zhang, Xiachen Lv, Li Zheng and Liang Ge. (2020). A translocation pathway for vesicle-mediated unconventional protein secretion. Cell 181:637-652.
2. Zhang, M., Kenny, S.J., Ge, L., Xu, K., and Schekman, R. (2015). Translocation of interleukin-1 β into a vesicle intermediate in autophagy-mediated secretion. eLife 4, e11205.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Zhang, M. and Liu, L. (2020). Proteinase K protection assay. Bio-protocol Preprint. [bio-protocol.org/prep398](https://doi.org/10.21203/rs.3.rs-45888/v1).
2. Zhang, M., Kenny, S. J., Ge, L., Xu, K. and Schekman, R. (2015). Translocation of interleukin-1 β into a vesicle intermediate in autophagy-mediated secretion. eLIFE. DOI: [10.7554/eLife.11205](https://doi.org/10.7554/eLife.11205)

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